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US-CL-CURRENT: 424/9.1,424/9.4 ,435/252.3 ,435/320.1 ,435/4 ,435/69.1 ,435/7.1 ,435/7.6 ,530/380 ,530/381

US-PAT-NO: 6121426
DOCUMENT-IDENTIFIER: US 6121426 A
TITLE: Fibrin binding domain polypeptides and uses and methods of producing same
DATE-ISSUED: September 19, 2000
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Vogel; Tikva	Rehovot	N/A	N/A	ILX
Levanon; Avigdor	Rehovot	N/A	N/A	ILX
Werber; Moshe	Tel Aviv	N/A	N/A	ILX
Guy; Rachel	Rehovot	N/A	N/A	ILX
Panet; Amos	Jerusalem	N/A	N/A	ILX

US-CL-CURRENT: 530/402,424/9.1 ,424/9.4 ,435/252.3 ,435/320.1 ,435/4 ,435/69.1 ,435/7.1 ,435/7.6 ,530/380 ,530/381

ABSTRACT:
This invention provides an imaging agent which comprises a polypeptide labeled with an imageable marker, such polypeptide having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin. The invention further provides a method wherein the imaging agent is used for imaging a fibrin-containing substance, i.e., a thrombus or atherosclerotic plaque. Further provided are plasmids for expression of polypeptides having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin, hosts containing these plasmids, methods of

producing the polypeptides, methods of treatment using the polypeptides, and methods of recovering, refolding and reoxidizing the polypeptides. The invention also provides for purified polypeptides substantially free of other substances of human origin which have an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and which are capable of binding to fibrin.

CLAIMS:

What is claimed is:

1. An imaging agent which comprises a polypeptide labeled with an imageable marker, wherein the polypeptide has a molecular weight between about 12 and about 20 kd, comprises an amino acid sequence identical to an amino acid sequence present in the N-terminal fibrin binding domain of naturally-occurring fibronectin the N-terminal sequence of which is glutamine-alanine-glutamine-glutamine and the length of which is sufficient to encompass the amino acid sequence of fibronectin required for binding to fibrin, and wherein the imaging agent is capable of binding to fibrin and wherein the marker is selected from the group consisting of indium-111, technetium-99m, iodine-123, iodine-131, krypton-81m, xenon-133, gallium-67 and paramagnetic ions.

2. A method for imaging a fibrin-containing substance which comprises contacting the fibrin-containing substance with the imaging agent of claim 1 under conditions such that the imaging agent binds to fibrin in the fibrin-containing substance, detecting the presence of any of imaging agent bound to fibrin and thereby imaging the fibrin-containing substance.

3. A method of claim 2, wherein the fibrin-containing substance is a thrombus.

4. A method of claim 2, wherein the fibrin-containing substance is atherosclerotic plaque.

5. The method of claim 2, wherein the fibrin-containing substance is within blood vessels of a subject and wherein contacting is performed by administering
the imaging agent contained in a suitable carrier to the subject under conditions permitting the imaging agent to enter the blood vessels of the subject.

6. A method of claim 5, wherein the fibrin-containing substance is a thrombus.

7. A method of claim 5, wherein the fibrin-containing substance is atherosclerotic plaque.

8. A method of claim 2, wherein the imaging is carried out using a gamma camera.

8 Claims, 95 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 86

US-CL-CURRENT: 530/300,530/317

US-PAT-NO: 6083481
DOCUMENT-IDENTIFIER: US 6083481 A
TITLE: Thrombus imaging agents
DATE-ISSUED: July 4, 2000
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dean; Richard T.	Bedford	NH	N/A	N/A
Lister-James; John	Bedford	NH	N/A	N/A

US-CL-CURRENT: 424/1.69,530/300 ,530/317
ABSTRACT:

This invention relates to radiolabeled reagents that are scintigraphic imaging agents for imaging sites of thrombus formation in vivo, and methods for producing such reagents. Specifically, the invention relates to reagents each comprised of a specific binding compound, capable of binding to at least one component of a thrombus, covalently linked to a radiolabel-binding moiety. The invention provides these reagents, methods and kits for making such reagents, and methods for using such reagents labeled with technetium-99m to image thrombus sites in a mammalian body.

CLAIMS:

What is claimed is:

1. A composition comprising technetium-99m and a reagent having a formula: ##STR12##

2. A complex having a structure:

3. A method of imaging a thrombus in a mammalian body comprising the steps of a) administering to said body an effective diagnostic amount of an imaging agent comprising technetium-99m and a reagent having a formula: ##STR13## and b) detecting technetium-99m localized at said thrombus.

3 Claims, 1 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 1

US-CL-CURRENT: 424/1.11,424/1.65 ,530/300 ,530/311 ,530/330 ,534/14

US-PAT-NO: 5968476
DOCUMENT-IDENTIFIER: US 5968476 A
TITLE: Technetium-99m labeled peptides for thrombus imaging
DATE-ISSUED: October 19, 1999
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dean; Richard T.	Bedford	NH	N/A	N/A
Lister-James; John	Bedford	NH	N/A	N/A

US-CL-CURRENT: 424/1.69,424/1.11 ,424/1.65 ,530/300 ,530/311 ,530/330 ,534/14

ABSTRACT:
This invention relates to radiolabeled peptides and methods for producing such peptides. Specifically, the invention relates to specific binding peptides, methods and kits for making such peptides, and methods for using such
peptides labeled with technetium-99m via a radiolabel-binding moiety covalently
linked to the peptide to image thrombus sites in a mammalian body.

CLAIMS:

What is claimed is:

1. A complex for thrombus imaging comprising technetium-99m complexed with a reagent comprising a peptide having an amino acid sequence of from 4 to 100 amino acids and a technetium-99m binding moiety covalently linked to the peptide, wherein the peptide is selected from the group consisting of a linear peptide ligand for a GPIIb/IIIa receptor not comprising the amino acid sequence
(arginine-glycine-asparate), a peptide ligand for a polymerization site of fibrin, and a cyclic peptide ligand for the GPIIb/IIIa receptor.

2. The complex of claim 1, formed by reacting the reagent with the technetium-99m in the presence of a reducing agent.

3. The complex of claim 2, wherein the reducing agent is selected from the group consisting of a dithionite ion, a stannous ion and a ferrous ion.

4. The complex of claim 1, formed by labeling the reagent with the technetium-99m by ligand exchange of a prereduced technetium-99m complex.

5. A method of labeling the reagent of claim 1, comprising the step of reacting the reagent with technetium-99m in the presence of a reducing agent.

6. The method of claim 5, wherein the reducing agent is selected from the group consisting of a dithionite ion, a stannous ion and a ferrous ion.

7. The complex of claim 1, wherein the peptide is a peptide ligand for a polymerization site of fibrin comprising multiple copies of the sequence (glycyl-prolyl-arginyl-prolyl).

8. The complex of claim 1, wherein the peptide comprises one of the following amino acid sequences:

(GPRPC.sub.Acm GC.sub.Acm C(S-maleimido)CH.sub.2 CH.sub.2 --).sub.3 N,

((GPRP).sub.2 K, or

(GPRVVERHQSA).sub.2 K.

9. The complex of claim 1, wherein the peptide comprises one of the following amino acid sequences: ##STR17##
9 Claims, 0 Drawing figures
Exemplary Claim Number: 1

US-CL-CURRENT: 424/1.69,424/9.34 ,424/9.4 ,424/9.42 ,435/252.3 ,435/320.1 ,435/4 ,435/7.1 ,435/7.6 ,530/380 ,530/381 ,530/402

US-PAT-NO: 5965383
DOCUMENT-IDENTIFIER: US 5965383 A
TITLE: Fibrin binding domain polypeptides and uses and methods of producing same
DATE-ISSUED: October 12, 1999
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Vogel; Tikva	Rehovot	N/A	N/A	ILX
Levanon; Avigdor	Rehovot	N/A	N/A	ILX
Werber; Moshe	Tel Aviv	N/A	N/A	ILX
Guy; Rachel	Rehovot	N/A	N/A	ILX
Panet; Amos	Jerusalem	N/A	N/A	ILX

US-CL-CURRENT: 435/69.1,424/1.69 ,424/9.34 ,424/9.4 ,424/9.42 ,435/252.3 ,435/320.1 ,435/4 ,435/7.1 ,435/7.6 ,530/380 ,530/381 ,530/402

ABSTRACT:

This invention provides an imaging agent which comprises a polypeptide labeled with an imageable marker, such polypeptide having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin. The invention further provides a method wherein the imaging agent is used for imaging a fibrin-containing substance, i.e., a thrombus-or atherosclerotic plaque. Further provided are plasmids for expression of polypeptides having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin, hosts containing these plasmids, methods of producing the polypeptides, methods of treatment using the polypeptides, and methods of recovering, refolding and reoxidizing the polypeptides. The invention also provides for purified polypeptides substantially free of other substances of human origin which have an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and which are capable of binding to fibrin.

CLAIMS:

What is claimed is:

1. An imaging agent which comprises a polypeptide labeled with an imageable marker, wherein the polypeptide has a molecular weight between about 12 and about 20 kD and an amino acid sequence substantially the same as an amino acid sequence present in the fibrin binding domain of naturally-occurring fibronectin, and having the amino acid sequence gln-ala-gln-gln or met-gln-ala-gln-gln at the N-terminus of the polypeptide and wherein the imaging agent is capable of binding to fibrin.

2. A composition comprising an effective imaging amount of the imaging agent of claim 1 and a physiologically acceptable carrier.

3. An agent of claim 1, wherein the marker is a radioactive isotope, an element which is opaque to X-rays, or a paramagnetic ion.

4. An agent of claim 3, wherein the marker is a radioactive isotope.

5. An agent of claim 4, wherein the radioactive isotope is indium-111.

6. An agent of claim 4, wherein the radioactive isotope is technetium-99m.

7. An agent of claim 4, wherein the radioactive isotope is iodine-123, iodine-125, iodine-131, krypton-81m, xenon-133, or gallium-67.

8. A purified polypeptide substantially free of other substances of human origin, wherein the polypeptide has a molecular weight between about 12 and about 20 kD and an amino acid sequence substantially the same as an amino acid sequence present in the fibrin binding domain of naturally-occurring human fibronectin, and having the amino acid sequence gln-ala-gln-gln or met-gln-ala-gln-gln at the N-terminus of the polypeptide, and is capable of binding to fibrin.

9. A plasmid for the expression of the polypeptide of claim 8 comprising DNA encoding the polypeptide and DNA encoding suitable regulatory elements so positioned relative to the DNA encoding the polypeptide as to express the polypeptide in a suitable host cell.

10. A cell which comprises the plasmid of claim 9.

11. A bacterial cell according to claim 10.

12. An Escherichia coli cell according to claim 11.

13. A method of producing a polypeptide having a molecular weight between about 12 and about 20 kD and an amino acid sequence substantially the same as an amino acid sequence present in the fibrin binding domain of naturally-occurring human fibronectin which comprises culturing a cell according to claim 10 so that the DNA expresses the polypeptide in the cell and
recovering from the cell the polypeptide so expressed.

14. A method for imaging a fibrin-containing substance which comprises contacting the fibrin-containing substance with the imaging agent of claim 1 under conditions such that the imaging agent binds to fibrin in the fibrin-containing substance, detecting the presence of any of imaging agent bound to fibrin and thereby imaging the fibrin-containing substance.

15. A method of claim 14, wherein the fibrin-containing substance is a thrombus.

16. A method of claim 14, wherein the fibrin-containing substance is atherosclerotic plaque.

17. The method of claim 14, wherein the fibrin-containing substance is within blood vessels of a subject and wherein contacting is performed by administering
the imaging agent contained in a suitable carrier to the subject under conditions permitting the imaging agent to enter the blood vessels of the subject.

18. A method of claim 17, wherein the fibrin-containing substance is a thrombus.

19. A method of claim 17, wherein the fibrin-containing substance is atherosclerotic plaque.

20. An agent of claim 14, wherein the marker is a radioactive isotope, an element which is opaque to X-rays, or a paramagnetic ion.

21. A method of claim 14, wherein the imaging is carried out using a gamma camera.
21 Claims, 86 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 75

US-CL-CURRENT: 530/331,530/380 ,530/382 ,534/14

US-PAT-NO: 5925331
DOCUMENT-IDENTIFIER: US 5925331 A
TITLE: Technetium-99m labeled peptides for thrombus imaging
DATE-ISSUED: July 20, 1999
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dean; Richard T.	Bedford	NH	N/A	N/A
Lister-James; John	Bedford	NH	N/A	N/A

US-CL-CURRENT: 424/1.69,530/331 ,530/380 ,530/382 ,534/14

ABSTRACT:
This invention relates to radiolabeled reagents that are scintigraphic imaging agents for imaging sites of thrombus formation in vivo, and methods for producing such reagents. Specifically, the invention relates to reagents each comprised of a specific binding compound, capable of binding to at least one component of a thrombus, covalently linked to a radiolabel-binding moiety. The invention provides these reagents, methods and kits for making such reagents, and methods for using such reagents labeled with technetium-99m to image thrombus sites in a mammalian body.

CLAIMS:

What is claimed is:

1. A reagent for preparing a scintigraphic imaging agent for imaging a thrombus within a mammalian body comprising a specific binding compound capable of binding to at least one component of a thrombus, covalently linked to a technetium-99m binding moiety, wherein the technetium-99m binding moiety has the formula:

C(pgp).sup.s -(aa)-C(pgp).sup.s

wherein C(pgp).sup.s is a cysteine having a protect thiol group and (aa) is an amino acid.

2. The reagent of claim 1 that is radiolabeled with technetium-99m.

3. The reagent of claim 1 wherein the specific binding compound and C(pgp).sup.s -(aa)-C(pgp).sup.s are covalently linked through from about one to about 20 amino acids.

4. The reagent of claim 1 wherein each of the protected cysteines comprising the technetium-99m binding moiety has a protecting group of the formula

--CH.sub.2 --NH--CO--R

wherein R is a lower alkyl having 1 to 6 carbon atoms, 2-,3-,4-pyridyl, phenyl,
or phenyl substituted with lower alkyl, hydroxy, lower alkoxy, carboxy, or lower alkoxycarbonyl.

5. The reagent of claim 1 wherein C(pgp).sup.s -(aa)-C(pgp).sup.s has the formula: ##STR14## .

6. The reagent of claim 1 wherein the specific binding compound is a peptide comprising 4 to 100 amino acids.

7. The reagent of claim 6 selected from the group consisting of: ##STR15## C.sub.Acm GC.sub.Acm GGRGDS, C.sub.Acm GC.sub.Acm GGRGDGGRGDS,

C.sub.Acm GC.sub.Acm GGRGDGGRGDGGRGDS,

C.sub.Acm GC.sub.Acm RRRRRRRRRGDV,

GRGDVKC.sub.Acm GC.sub.Acm.amide,

GRGDVC.sub.Acm GC.sub.Acm.amide,

GRGDVRGDFKC.sub.Acm GC.sub.Acm.amide,

GRGDVRGDFC.sub.Acm GC.sub.Acm.amide,

acetyl-G.Apc.GDV.Apc.GDFKC.sub.Acm GC.sub.Acm.amide,

G.Apc.GCV.Apc.GDFKC.sub.Acm GC.sub.Acm.amide,

G.Aklpc.GDVKC.sub.Acm GC.sub.Acm.amide,

(CC.sub.Acm GC.sub.Acm GGRGDS).sub.3 --TSEA,

C.sub.Acm GC.sub.Acm NDGDFEEIPEEYLQ,

C.sub.Acm GC.sub.Acm GGF.sub.D PRPGGGGNGDFEEIPEEYL,

C.sub.Acm GC.sub.Acm GGF.sub.D PRPGamide,

[(GPRP).sub.2 K].sub.2 KC.sub.Acm GC.sub.Acm.amide,

(GPRVVERHQSA).sub.2 KC.sub.Acm GC.sub.Acm.amide,

(GPRPC.sub.Acm GC.sub.Acm C).sub.3 --TSEA,

[GPRPPPGGC.sub.Acm GC.sub.Acm GGC].sub.3 --TSEA, ##STR16## acetyl-RRARGDDLDC.sub.Acm GC.sub.Acm.amide, and PSPSPIHPAHHKRDRRQC.sub.Acm GC.sub.Acm.amide.

8. A complex formed by reacting the reagent of claim 1 with technetium-99m in the presence of a reducing agent.

9. The complex of claim 8, wherein the reducing agent is selected from the group consisting of a dithionite ion, a stannous ion and a ferrous ion.

10. A complex formed by labeling the reagent of claim 1 with technetium-99m by
ligand exchange of a prereduced technetium-99m complex.

11. A kit for preparing a radiopharmaceutical preparation, said kit comprising
a sealed vial containing a predetermined quantity of the reagent of claim 1 and
a sufficient amount of reducing agent to label the reagent with technetium-99m.

12. A method for labeling a reagent according to claim 1 comprising reacting the reagent with technetium-99m in the presence of a reducing agent.

13. The method of claim 12, wherein the reducing agent is selected from the group consisting of a dithionite ion, a stannous ion and a ferrous ion.

14. A method for imaging a thrombus within a mammalian body comprising the steps of administering an effective diagnostic amount of the technetium-99m radiolabeled reagent of claim 2 to an animal and detecting the radiolabeled reagent localized at the site of a thrombus.

15. The reagent according to claim 1 wherein the specific-binding peptide is chemically synthesized in vitro.

16. The reagent according to claim 15 wherein the specific-binding peptide is synthesized by solid phase peptide synthesis.

17. The reagent according to claim 15 wherein the radiolabel-binding moiety is
covalently linked to the specific-binding peptide during in vitro chemical synthesis.

18. The reagent according to claim 17 wherein the radiolabel-binding moiety is
covalently linked to the specific-binding peptide during solid phase peptide synthesis.

19. A composition of matter comprising a reagent selected from the group consisting of: ##STR17## C.sub.Acm GC.sub.Acm GGRGDS, C.sub.Acm GC.sub.Acm GGRGDGGRGDS,

C.sub.Acm GC.sub.Acm GGRGDGGRGDGGRGDS,

C.sub.Acm GC.sub.Acm RRRRRRRRRGDV,

GRGDVKC.sub.Acm GC.sub.Acm.amide,

GRGDVC.sub.Acm GC.sub.Acm.amide,

GRGDVRGDFKC.sub.Acm GC.sub.Acm.amide,

GRGDVRGDFC.sub.Acm GC.sub.Acm.amide,

acetyl-G.apc.GDV.Apc.GDFKC.sub.Acm GC.sub.Acm.amide,

G.Apc.GDV.Apc.GDFKC.sub.Acm GC.sub.Acm.amide,

G.Apc.GDVKC.sub.Acm GC.sub.Acm.amide,

(CC.sub.Acm GC.sub.Acm GGRGDS).sub.3 --TSEA,

C.sub.Acm GC.sub.Acm NDGDFEEIPEEYLQ,

C.sub.Acm GC.sub.Acm GGF.sub.D PRPGGGGNGDFEEIPEEYL,

C.sub.Acm GC.sub.Acm GGF.sub.D PRPGamide,

[(GPRP).sub.2 K].sub.2 KC.sub.Acm GC.sub.Acm.amide,

(GPRVVERHQSA).sub.2 KC.sub.Acm GC.sub.Acm.amide,

(GPRPC.sub.Acm GC.sub.Acm C).sub.3 --TSEA, ##STR18## .

20. The reagent of claim 1 wherein the reagent further comprises a polyvalent linking moiety covalently linked to a multiplicity of specific binding compounds and also covalently linked to a multiplicity of radiolabel-binding moieties to comprise a reagent for preparing a multimeric polyvalent scintigraphic imaging agent; wherein the molecular weight of the multimeric polyvalent scintigraphic imaging agent is less than about 20,000 daltons.

21. The reagent of claim 20 wherein the polyvalent linking moiety is bis-succinimidylmethylether, 4-(2,2-dimethylacetyl)benzoic acid, N-[2-(N',N'-bis(2-succinimidoethyl)aminoethyl)]--N.sup.6,N.sup.9 -bis(2-methyl-2-mercaptopropyl)-6,9-diazanonanamide, tris(succinimidylethyl)amine, bis-succinimidohexane, or 4-(O--CH.sub.2 CO--Gly--Gly--Cys.amide)acetophenone.

22. A reagent for preparing a thrombus imaging agent for imaging a thrombus within la mammalian body comprising a specific binding peptide having an amino acid sequence of 40 to 100 amino acids and a technetium-99m binding moiety covalently linked to the specific binding peptide, wherein the peptide is a ligand for a polymerization site of fibrin, having an amino acid sequence comprising multiple copies of the sequence (glycyl-prolyl-arginyl-prolyl).

23. A reagent for preparing a thrombus imaging agent for imaging a thrombus within a mammalian body comprising a specific binding peptide having an amino acid sequence of 4 to 100 amino acids and a technetium-99m binding moiety covalently linked to the specific binding peptide, wherein the reagent has the formula:

[(GPRP).sub.2 K].sub.2 KC.sub.Acm GC.sub.Acm.amide,

(GPRVVERHQSA).sub.2 KC.sub.Acm GC.sub.Acm.amide,

(GPRPC.sub.Acm GC.sub.Acm C).sub.3 --TSEA,

[GPRPPPGGC.sub.Acm GC.sub.Acm GGC].sub.3 --TSEA,

acetyl-CNP.Apc.GDC,

[BAT].Hly.GDP.Hly.GDF.amide,

[BAT]G.Apc.GDV.Apc.GDFK.amide, ##STR19## acetyl-G.Apc.GDV.Apc.GDFKC.sub.Acm GC.sub.Acm.amide, G.Apc.GDV.Apc.GDFKC.sub.Acm GC.sub.Acm.amide, or

G.Apc.GDVKC.sub.Acm GC.sub.Acm.amide.

24. The reagent of claim 22, that is radiolabled with technetium-99m.

25. The reagent according to claim 22 wherein the specific-binding peptide and
the technetium-99m binding moiety are covalently linked through from about to about 20 amino acids.

26. A complex formed by reacting the reagent according to claim 22 with technetium-99m in the presence of a reducing agent.

27. The complex of claim 26, wherein the reducing agent is selected from the group consisting of a dithionite ion, a stannous ion and a ferrous ion.

28. A complex formed by labeling the reagent according to claim 22 with technetium-99m by ligand exchange of a prereduced technetium-99m complex.

29. A composition of matter comprising the reagent according to claim 22 and a
stannous ion.

30. A kit for preparing a radiopharmaceutical preparation, said kit comprising
a sealed vial containing a predetermined quantity of a reagent according to claim 22 and a sufficient amount of reducing agent to label said reagent with technetium-99m.

31. A method for labeling a reagent according to claim 22 comprising reacting the reagent with technetium-99m in the presence of a reducing agent.

32. The method of claim 31, wherein the reducing agent is selected from the group consisting of a dithionite ion, a stannous ion and a ferrous ion.

33. A method for imaging a thrombus within a mammalian body comprising the steps of administering an effective diagnostic amount of the technetium-99m radiolabeled reagent of claim 26 to an animal and detecting the radiolabeled reagent localized at the site of a thrombus.

34. The reagent according to claim 22 wherein the specific-binding peptide is chemically synthesized in vitro.

35. The specific-binding peptide according to claim 34 wherein the peptide is synthesized by solid phase peptide synthesis.

36. The reagent according to claim 34 wherein the technetium-99m binding

moiety is covalently linked to the peptide during in vitro chemical synthesis.

37. The reagent according to claim 36, wherein the technetium-99m binding moiety is covalently linked to the peptide during solid phase peptide synthesis.

38. The reagent of claim 22 wherein the reagent further comprises a polyvalent
linking moiety covalently linked to a multiplicity of specific binding compounds and also covalently linked to a multiplicity of radiolabel-binding moieties to comprise a reagent for preparing a multimeric polyvalent scintigraphic imaging agent, wherein the molecular weight of the multimeric polyvalent scintigraphic imaging agent is less than about 20,000 daltons.

39. The reagent of claim 38 wherein the polyvalent linking moiety is bis-succinimidylmethylether, 4-(2,2-dimethylacetyl)benzoic acid, N-[2-(N',N'-bis(2-succinimidoethyl)aminoethyl)]--N.sup.6,N.sup.9 -bis(2-methyl-2-mercaptopropyl)-6,9-diazanonanamide, tris(succinimidylethyl)amine, bis-succinimidohexane, or 4-(O--CH.sub.2 CO--Gly--Gly--Cys.amide)acetophenone.

39 Claims, 1 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 1

US-CL-CURRENT: 424/1.11,424/1.65 ,530/300 ,534/14

US-PAT-NO: 5888474
DOCUMENT-IDENTIFIER: US 5888474 A
TITLE: Technetium-99m labeled peptides for GPIIb/IIIa ligands useful for thrombus imaging
DATE-ISSUED: March 30, 1999
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dean; Richard T.	Bedford	NH	N/A	N/A
Lister-James; John	Bedford	NH	N/A	N/A

US-CL-CURRENT: 424/1.69,424/1.11 ,424/1.65 ,530/300 ,534/14
ABSTRACT:
This invention relates to radiolabeled peptides and methods for producing such peptides. Specifically, the invention relates to specific binding peptides, methods and kits for making such peptides, and methods for using such
peptides labeled with technetium-99m via a radiolabel-binding moiety covalently
linked to the peptide to image thrombus sites in a mammalian body.

CLAIMS:

What is claimed is:

1. A reagent for preparing a thrombus imaging agent comprising a specific binding peptide having an amino acid sequence of 4 to 100 amino acids and a technetium-99m binding moiety covalently linked to the peptide, wherein the peptide is selected from the group consisting of a cyclic peptide ligand for a GPIIb/IIIa receptor and a linear peptide ligand for the GPIlb/IIIa receptor not
comprising the amino acid sequence (arginine-glycine-aspartate).

2. A composition comprising a peptide having a formula selected from the group
consisting of: ##STR17##

3. The reagent according to claim 1, wherein the peptide and the technetium-99m binding moiety are covalently linked through one or more amino acids.

4. A composition of matter comprising the reagent according to claim 1 and a stannous ion.

5. A kit for preparing a radiopharmaceutical preparation, said kit comprising a sealed vial containing a predetermined quantity of a reagent according to claim 1 and a sufficient amount of a reducing agent to label said reagent with technetium-99m.

6. The reagent according to claim 1, wherein the peptide is chemically synthesized in vitro.

7. The reagent according to claim 6, wherein the peptide is synthesized by solid phase peptide synthesis.

8. The reagent according to claim 6 wherein the technetium-99m binding moiety is covalently linked to the peptide during in vitro chemical synthesis.

9. The reagent according to claim 8 wherein the technetium-99m binding moiety is covalently linked to the peptide during solid phase peptide synthesis.

10. The reagent of claim 1, wherein the peptide is selected from the group consisting of: ##STR18##
10 Claims, 0 Drawing figures
Exemplary Claim Number: 1

US-CL-CURRENT: 424/9.341,424/9.4 ,530/381 ,530/382 ,530/395

US-PAT-NO: 5869616
DOCUMENT-IDENTIFIER: US 5869616 A
TITLE: Fibrin binding domain polypeptides and uses and methods of producing same
DATE-ISSUED: February 9, 1999
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Vogel; Tikva	Rehovot	N/A	N/A	ILX
Levanon; Avigdor	Rehovot	N/A	N/A	ILX
Werber; Moshe M.	Tel Aviv	N/A	N/A	ILX
Guy; Rachel	Rehovot	N/A	N/A	ILX
Panet; Amos	Jerusalem	N/A	N/A	ILX
Hartman; Jacob	Holon	N/A	N/A	ILX
Shaked; Hadassa	Ramat Gan	N/A	N/A	ILX

US-CL-CURRENT: 530/380,424/9.341 ,424/9.4 ,530/381 ,530/382 ,530/395
ABSTRACT:
This invention provides an imaging agent which comprises a polypeptide labeled with an imageable marker, such polypeptide having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin. The invention further provides a method wherein the imaging agent is used for imaging a fibrin-containing substance, i.e., a thrombus or atherosclerotic plaque. Further provided are plasmids for expression of polypeptides having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin, hosts containing these plasmids, methods of producing the polypeptides, methods of treatment using the polypeptides, and methods of recovering, refolding and reoxidizing the polypeptides. The invention also provides for purified polypeptides substantially free of other substances of human origin which have an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and which are capable of binding to fibrin.

CLAIMS:

What is claimed is:

1. An imaging agent which comprises a polypeptide labeled with an imageable marker, such polypeptide having an amino acid sequence substantially identical to a sequence present in the fibrin binding domain of naturally-occurring human fibronectin, being capable of binding to fibrin, having a molecular weight above about 6 kD but less than about 20 kD, and having the amino acid sequence gln-ala-gln-gln (SEQ ID NO: 1) or met-gln-ala-gln-gln (SEQ ID NO: 8) at the N-terminus of the polypeptide.

2. An imaging agent of claim 1, wherein the polypeptide has a molecular weight of about 12 kD or above.

3. An imaging agent of claim 1, wherein the polypeptide has a molecular weight
of about 20 kD or below.

4. An imaging agent of claim 1, wherein the polypeptide has a molecular weight
of about 18.5 kD or below.

5. A composition comprising an effective imaging amount of the imaging agent of claim 1 and a physiologically acceptable carrier.

6. An agent of claim 1, wherein the marker is a radioactive isotope, an element which is opaque to X-rays, or a paramagnetic ion.

7. An agent of claim 6, wherein the marker is a radioactive isotope.

8. An agent of claim 7, wherein the radioactive isotope is indium-111.

9. An agent of claim 7, wherein the radioactive isotope is technetium-99m.

10. An agent of claim 7, wherein the radioactive isotope is iodine-123, iodine-125, iodine-131, krypton-81m, xenon-133, or gallium-67.

11. An agent of claim 1, wherein the polypeptide comprises a 20 kD polypeptide
wherein the amino acid sequence substantially identical to a sequence present in the fibrin binding domain of human fibronectin is the amino acid sequence of
amino acids 1-153 as shown in FIG. 2 (SEQ ID NO. 16).

12. An agent of claim 11, wherein the polypeptide comprises less than about 20
additional amino acids.

13. An agent of claim 1, wherein the polypeptide is an 18.5 kD polypeptide wherein the amino acid sequence substantially identical to a sequence present in the fibrin binding domain of human fibronectin is the sequence of amino acids 1-154 as shown in FIG. 2 (SEQ ID NO: 16).

14. A purified polypeptide substantially free of other substances of human origin which has an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin, is the same as that capable of binding to fibrin, has a molecular weight above 6 kD but less than 20 kD, and has the amino acid sequence gln-ala-gln-gln or met-gln-ala-gln-gln at its N-terminus.

15. A polypeptide of claim 14, wherein the polypeptide comprises a 20 kD polypeptide corresponding to an amino acid sequence present in the fibrin binding domain of human fibronectin, having the amino acid sequence of amino acids 1-153 as shown in FIG. 2 (SEQ ID NO: 16) and having the amino acid sequence gln-ala-gln-gln or met-gln-ala-gln-gln at the N-terminus of the polypeptide.

16. A polypeptide of claim 15, wherein the polypeptide comprises less than about 20 additional amino acids.

17. A polypeptide of claim 14, wherein the polypeptide is a 12 kD polypeptide corresponding to an amino acid sequence present in the fibrin binding domain of
human fibronectin, having the amino acid sequence of amino acids 1-109 as shown
in FIG. 2 (SEQ ID NO: 16) and having the amino acid sequence gln-ala-gln-gln (SEQ ID NO: 1) or met-gln-ala-gln-gln (SEQ ID NO: 8) at the N-terminus of the polypeptide.

18. A polypeptide of claim 14, wherein the polypeptide is an 18.5 kD polypeptide corresponding to an amino acid sequence present in the fibrin binding domain of human fibronectin, having the amino acid sequence of amino acids 1-154 as shown in FIG. 2 (SEQ ID NO: 16) and having the amino acid sequence gln-ala-gln-gln (SEQ ID NO: 1) or met-gln-ala-gln-gln (SEQ ID NO: 8) at the N-terminus of the polypeptide.

18 Claims, 82 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 66

US-CL-CURRENT: 424/9.1,435/69.6 ,514/8 ,530/350 ,530/402

US-PAT-NO: 5792742
DOCUMENT-IDENTIFIER: US 5792742 A
TITLE: Fibrin-binding peptide fragments of fibronectin
DATE-ISSUED: August 11, 1998
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gold; Leslie I.	New York	NY	N/A	N/A
Rostagno; Agueda A.	Elmhurst	NY	N/A	N/A
Baron; Martin	Oxford	N/A	N/A	GBX
Campbell; Iain D.	Oxford	N/A	N/A	GBX
Williams; Michael J.	Oxford	N/A	N/A	GBX

US-CL-CURRENT: 514/2,424/9.1 ,435/69.6 ,514/8 ,530/350 ,530/402

ABSTRACT:

Fibrin-binding molecules are provided which include at least one peptide essentially corresponding to one or both of the following portions of the natural fibronectin molecule. The first portion is that portion which includes
the .sup.4 F1..sup.5 F1 module pair of fibronectin and includes no more of the natural fibronectin molecule than the N-terminal 25.9 kDa proteolytic fragment.
The second portion includes the .sup.10 F1..sup.11 F1 module pair of fibronectin and includes no more of the natural fibronectin molecule than the C-terminal 11 kDa proteolytic fragment. Also disclosed are nucleic acid molecules encoding the fibrin-binding peptides, methods for making the peptides, methods for using the peptides in the diagnosis and treatment of cardiovascular, peripheral vascular, cerebrovascular, and other conditions associated with fibrin deposition, and assay methods for detecting a fibrin-binding molecule and for measuring fibrin.

CLAIMS:

What is claimed is:

1. A fibrin-binding molecule including at least one peptide selected from the group consisting of:

a) a portion of the natural fibronectin molecule which includes positions 150-244 of SEQ ID NO:1, wherein the fibrin-binding molecule includes no more of
the N-terminal portion of the natural fibronectin molecule than the N-terminal 25.9 kDa proteolytic fragment thereof; and

b) a portion of the natural fibronectin molecule which includes positions 2122-2232 of SEQ ID NO:1, wherein the fibrin-binding molecule includes no more of the C-terminal portion of the natural fibronectin molecule than the C-terminal 11 kDa proteolytic thereof.

2. A fibrin-binding molecule in accordance with claim 1, wherein said molecule

includes peptide a) but not peptide b).

3. A fibrin-binding molecule according to claim 2 wherein said molecule includes a peptide corresponding to an N-terminal portion of fibronectin having
an apparent molecular weight of about 25.9 kDa as determined by laser desorption mass spectrometry.

4. A fibrin-binding molecule in accordance with claim 1, wherein said molecule
includes peptide b) but not peptide a).

5. A fibrin-binding molecule according to claim 4, wherein said molecule includes a peptide corresponding to a C-terminal portion of fibronectin having an apparent molecular weight of about 11 kDa on sodium dodecyl sulfate polyacrylamide gel electrophoresis.

6. A fibrin-binding molecule in accordance with claim 1, wherein said molecule
includes both peptide a) and peptide b).

7. A fibrin-binding molecule in accordance with claim 1, selected from the group consisting of:

(a) the portion of fibronectin corresponding to positions 150-244 of SEQ ID NO:1; and

(b) the portion of fibronectin corresponding to positions 2122-2232 of SEQ ID NO:1.

8. A fibrin-binding molecule in accordance with claim 1, further including, bound to said peptide, a therapeutic agent or a diagnostic marker.

9. A molecule according to claim 8, wherein said peptide is labeled with a detectable label.

10. A molecule according to claim 8, wherein said peptide is conjugated to a therapeutic agent.

11. A molecule according to claim 10, wherein said therapeutic agent is selected from a thrombolytic and a fibrinolytic agent.

12. A molecule according to claim 10, wherein said therapeutic agent is a cytotoxic agent.

13. A pharmaceutical composition, comprising a fibrin-binding molecule

according to 1, and a pharmaceutically acceptable carrier.
13 Claims, 57 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 33

US-CL-CURRENT: 514/2,530/380 ,530/381 ,530/382

US-PAT-NO: 5679320
DOCUMENT-IDENTIFIER: US 5679320 A
TITLE: Fibrin binding domain polypeptides and uses and methods of producing same
DATE-ISSUED: October 21, 1997
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Vogel; Tikva	Rehovot	N/A	N/A	ILX
Levanon; Avigdor	Rehovot	N/A	N/A	ILX
Werber; Moshe M.	Tel Aviv	N/A	N/A	ILX
Guy; Rachel	Rehovot	N/A	N/A	ILX
Panet; Amos	Jerusalem	N/A	N/A	ILX
Hartman; Jacob	Holon	N/A	N/A	ILX
Shaked; Hadassa	Ramat Gan	N/A	N/A	ILX

US-CL-CURRENT: 424/1.69,514/2 ,530/380 ,530/381 ,530/382
ABSTRACT:
This invention provides an imaging agent which comprises a polypeptide labeled with an imageable marker, such polypeptide having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin. The invention further provides a method wherein the imaging agent is used for imagifig a fibrin-containing substance, i.e., a thrombus or atherosclerotic plaque. Further provided are plasmids for expression of polypeptides having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin, hosts containing these plasmids, methods of producing the polypeptides, methods of treatment using the polypeptides, and methods of recovering, refolding and reoxidizing the polypeptides. The invention also provides for purified polypeptides substantially free of other substances of human origin which have an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and which are capable of binding to fibrin.

CLAIMS:

What is claimed is:

1. A method for imaging a fibrin-containing substance which comprises contacting the fibrin-containing substance to be imaged with an imaging agent which comprises a polypeptide labeled with an imageable marker, such polypeptide havinq an amino acid sequence substantially identical to a sequence present in the fibrin binding domain of naturally occurrinq human fibronectin, beinq capable of binding to fibrin, having a molecular weiqht above about 6 kD but less than about 20 kD, and having the amino acid sequence gln-ala-gln-gln or met-gln-ala-gln-gln at the N-terminus of the polypeptide under conditions such that the agent binds to the fibrin-containing substance and imaging bound agent and thereby imaging the fibrin-containing substance.

2. A method of claim 1, wherein the fibrin-containing substance is a thrombus.

3. A method of claim 1, wherein the fibrin-containing substance is atherosclerotic plaque.

4. A method for imaging a fibrin-containing substance in a subject which comprises:

(a) administering to the subject a composition comprising an effective imaging amount of the imaging agent used in claim 1 and a physiologically. acceptable carrier under conditions permitting the imaging agent container therein to enter the blood stream and bind to fibrin present in the blood vessels;

(b) imaging bound agent within the blood vessels; and thereby

(c) imaging the fibrin-containing substance.

5. A method of claim 4, wherein the fibrin-containing substance is a thrombus.

6. A method of claim 4, wherein the fibrin-containing substance is atherosclerotic plaque.

7. A method of claim 1, wherein the polypeptide is an 18.5 kD polypeptide wherein the amino acid sequence substantially identical to a sequence present in the fibrin binding domain of human fibronectin is the sequence of amino acids 1-154 as shown in FIG. 2.

8. A method of claim 16, wherein the polypeptide comprises a 20 kD polypeptide
wherein the amino acid sequence substantially identical to a sequence present in the fibrin binding domain of human fibronectin is the sequence of amino acids 1-153 as shown in FIG. 2 (SEQ ID NO. 16).

9. A method of claim 8, wherein the 20 kD polypeptide comprises less than about 20 additional amino acids to the C-terminal of the sequence of amino acids 1-153 as shown in FIG. 2A.

10. A method of claim 1, wherein the polypeptide is a 12 kD polypeptide wherein the amino acid sequence substantially identical to a sequence present in the fibrin binding domain of human fibronectin is the sequence of amino acids 1-109 as shown in FIG. 2 (SEQ ID NO. 16).

11. A method of claim 1, wherein the marker is a radioactive isotope, an element which is opaque to X-rays, or a paramagnetic ion.

12. A method of claim 1, wherein the imaging is carried out using a gamma camera.

13. A method for imaging a fibrin-containing substance which comprises contacting the fibrin-containing substance to be imaged with an imaging agent which comprises a polypeptide labeled with an imageable marker, such polypeptide comprising a 12 kD amino acid sequence encoded by plasmid pFN 203-2
(ATCC 68606), being capable of binding to fibrin, and having a molecular weight
from about 12 kD up to about 20 kD, and having the amino acid sequence gln-ala-gln-gln or met-gln-ala-gln-gln at the N-terminus of the potypeptide under conditions such that the agent binds to the fibrin-containing substance and imaging bound agent and thereby imaging the fibrin-containing substance.
13 Claims, 82 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 66

US-CL-CURRENT: 424/1.69,424/9.341 ,424/9.4 ,435/13 ,435/7.8 ,436/503 ,436/504 ,436/69

US-PAT-NO: 5455158
DOCUMENT-IDENTIFIER: US 5455158 A
TITLE: Fibrin binding domain polypeptides and uses and methods of producing same
DATE-ISSUED: October 3, 1995
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Vogel; Tikva	Rehovot	N/A	N/A	ILX
Levanon; Avigdor	Rehovot	N/A	N/A	ILX
Werber; Moshe M.	Tel Aviv	N/A	N/A	ILX
Guy; Rachel	Rehovot	N/A	N/A	ILX
Panet; Amos	Jerusalem	N/A	N/A	ILX

US-CL-CURRENT: 435/7.21,424/1.69 ,424/9.341 ,424/9.4 ,435/13 ,435/7.8 ,436/503 ,436/504 ,436/69

ABSTRACT:
This invention provides an imaging agent which comprises a polypeptide labeled with an imageable marker, such polypeptide having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin. The invention further provides a method wherein the imaging agent is used for imaging a fibrin-containing substance, i.e., a thrombus or atherosclerotic plaque. Further provided are plasmids for expression of polypeptides having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin, hosts containing these plasmids, methods of producing the polypeptides, methods of treatment using the polypeptides, and methods of recovering, refolding and reoxidizing the polypeptides. The invention also provides for purified polypeptides substantially free of other substances of human origin which have an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and which are capable of binding to fibrin.

CLAIMS:

What is claimed is:

1. A method for imaging a fibrin-containing substance which comprises

contacting the fibrin-containing substance to be imaged with an imaging agent under conditions such that the imaging agent binds to fibrin in the fibrin-containing substance,

imaging bound imaging agent, and

thereby imaging the fibrin-containing substance,

wherein the imaging agent comprises a polypeptide labeled with an imageable marker,

wherein the polypeptide is a 12 kD polypeptide corresponding to an amino acid sequence present in the fibrin binding domain of naturally-occurring human fibronectin and comprising the amino acid sequence of amino acids 1-109 as shown in FIG. 1.

2. A method of claim 1, wherein the fibrin-containing substance is a thrombus.

3. A method of claim 1, wherein the fibrin-containing substance is atherosclerotic plaque.

4. The method according to claim 1 wherein the fibrin-containing substance is within blood vessels of a subject and wherein contacting is performed by administering the imaging agent contained in a suitable carrier to the subject under conditions permitting the imaging agent to enter the blood vessels of the
subject.

5. A method of claim 4, wherein the fibrin-containing substance is a thrombus.

6. A method of claim 4, wherein the fibrin-containing substance is atherosclerotic plaque.

7. A method of claim 1, wherein the marker is a radioactive isotope, an element which is opaque to X-rays, or a paramagnetic ion.

8. A method of claim 1, wherein the imaging is carried out using a gamma camera.

8 Claims, 98 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 86

US-PAT-NO: 5440014
DOCUMENT-IDENTIFIER: US 5440014 A
TITLE: Fibronectin binding peptide
DATE-ISSUED: August 8, 1995
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hook; Magnus	Birmingham	AL	35244	N/A
McGavin; Martin	Birmingham	AL	35209	N/A
Raucci; Guiseppe	I-00040 Pomezia, Rome	N/A	N/A	ITX

US-CL-CURRENT: 530/326

ABSTRACT:
A fibronectin binding peptide having the structure R'-PSYQFGGHNS VDFEEDT-R.sup.2 wherein R' is hydrogen, K or DK, and R.sup.2 is hydroxy, L, LP or LPK is disclosed. The fibronectin binding proteins of the present invention
may be used, for example, for vaccination of ruminants against mastitis caused by Staphylococcal infections, for the treatment of wounds, e.g., for blocking protein receptors or for immunization (vaccination) against infection by bacterial strains, and for diagnosis of bacterial infections caused by Staphylococci strains.

CLAIMS:

We claim:

1. Fibronectin binding peptide consisting of the structure [SEQ ID NO. 1]R'-PSYQFGGHNS VDFEEDT-R.sup.2 wherein R' is hydrogen, K or DK, and R.sup.2 is
hydroxy, L, LP or LPK.

1 Claims, 6 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 6

US-CL-CURRENT: 424/9.341,424/9.4 ,435/252.3 ,435/252.33 ,435/320.1 ,435/69.1 ,530/350 ,530/395

US-PAT-NO: 5270030
DOCUMENT-IDENTIFIER: US 5270030 A
TITLE: Fibrin binding domain polypeptide and method of producing
DATE-ISSUED: December 14, 1993
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Vogel; Tikva	Rehovot	N/A	N/A	ILX
Levanon; Avigdor	Rehovot	N/A	N/A	ILX
Werber; Moshe M.	Tel Aviv	N/A	N/A	ILX
Guy; Rachel	Rehovot	N/A	N/A	ILX
Panet; Amos	Jerusalem	N/A	N/A	ILX

US-CL-CURRENT: 424/1.69,424/9.341 ,424/9.4 ,435/252.3 ,435/252.33 ,435/320.1 ,435/69.1 ,530/350 ,530/395

ABSTRACT:
This invention provides an imaging agent which comprises a polypeptide labeled with an imageable marker, such polypeptide having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin. The invention further provides a method wherein the imaging agent is used for imaging a fibrin-containing substance, i.e., a thrombus or atherosclerotic plaque. Further provided are plasmids for expression of polypeptides having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin, hosts containing these plasmids, methods of producing the polypeptides, methods of treatment using the polypeptides, and methods of recovering, refolding and reoxidizing the polypeptides. The invention also provides for purified polypeptides substantially free of other substances of human origin which have an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and which are capable of binding to fibrin.

CLAIMS:

What is claimed is:

1. An imaging agent which comprises a polypeptide labeled with an imageable marker, wherein the polypeptide is a 12 kD polypeptide corresponding to an amino acid sequence present in the fibrin binding domain of naturally-occurring human fibronectin and having the amino acid sequence of amino acids 1-109 as shown in FIG. 1 and being capable of binding to fibrin.

2. A composition comprising an effective imaging amount of the imaging agent of claim 1 and a physiologically acceptable carrier.

3. An agent of claim 1, wherein the marker is a radioactive isotope, an element which is opaque to X-rays, or a paramagnetic ion.

4. An agent of claim 3, wherein the marker is a radioactive isotope.

5. An agent of claim 4, wherein the radioactive isotope is indium-111.

6. An agent of claim 4, wherein the radioactive isotope is technetium-99m.

7. An agent of claim 4, wherein the radioactive isotope is iodine-123, iodine-125, iodine-131, krypton-81m, xenon-133, or gallium-67.

8. A purified polypeptide substantially free of other substances of human origin wherein the polypeptide is a 12 kD polypeptide of amino acids 1-109 as shown in FIG. 1 corresponding to an amino acid sequence present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin.

9. A plasmid for expression of the polypeptide of claim 8 comprising DNA encoding the polypeptide and DNA encoding suitable regulatory elements positioned relative to the DNA encoding the polypeptide so as to effect expression of the polypeptide in a suitable host cell.

10. A plasmid according to claim 9 designated pFN 196-2 and deposited in escherichia coli strain A4255 under ATCC Accession No. 63328.

11. A cell which comprises the plasmid of claim 9.

12. A bacterial cell according to claim 11.

13. An Escherichia coli cell according to claim 12.

14. An Escherichia coli cell according to claim 13, wherein the plasmid is designated pFN 196-2 and wherein the cell is deposited under ATCC Acession No. 68328.

15. A method of producing a 12 kD polypeptide fragment corresponding to an amino acid sequence present in the fibrin binding domain of naturally-occurring
human fibronectin which comprises culturing a cell according to claim 11 so that the DNA directs expression of the polypeptide and the cell expressed the polypeptide and recovering from the cell the polypeptide so expressed.
15 Claims, 62 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 86

CLIPPEDIMAGE= US005869616A
PUB-NO: US005869616A
DOCUMENT-IDENTIFIER: US 5869616 A
TITLE: Fibrin binding domain polypeptides and uses and methods of producing same
PUBN-DATE: February 9, 1999
INVENTOR-INFORMATION:

NAME	COUNTRY
VOGEL, TIKVA	IL
LEVANON, AVIGDOR	IL
WERBER, MOSHE M	IL
GUY, RACHEL	IL
PANET, AMOS	IL
HARTMAN, JACOB	IL
SHAKED, HADASSA	IL

EUR-CL (EPC): A61K051/08; A61K049/00 ABSTRACT:
This invention provides an imaging agent which comprises a polypeptide labeled with an imageable marker, such polypeptide having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human
fibronectin and being capable of binding to fibrin. The invention further provides a method wherein the imaging agent is used for imaging a fibrin-containing substance, i.e., a thrombus or atherosclerotic plaque. Further provided are plasmids for expression of polypeptides having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin, hosts containing these plasmids, methods of producing the polypeptides, methods
of treatment using the polypeptides, and methods of recovering, refolding and reoxidizing the polypeptides. The invention also provides for purified polypeptides substantially free of other substances of human origin which have an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and which are capable of binding to fibrin.
INT-CL (IPC): C07K014/745; A61K051/08
ABSTRACT:
This invention provides an imaging agent which comprises a polypeptide labeled with an imageable marker, such polypeptide having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human
fibronectin and being capable of binding to fibrin. The invention further provides a method wherein the imaging agent is used for imaging a fibrin-containing substance, i.e., a thrombus or atherosclerotic plaque. Further provided are plasmids for expression of polypeptides having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin, hosts containing these plasmids, methods of producing the polypeptides, methods
of treatment using the polypeptides, and methods of recovering, refolding and reoxidizing the polypeptides. The invention also provides for purified polypeptides substantially free of other substances of human origin which have an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and which are capable of binding to fibrin.

CLIPPEDIMAGE= US005679320A
PUB-NO: US005679320A
DOCUMENT-IDENTIFIER: US 5679320 A
TITLE: Fibrin binding domain polypeptides and uses and methods of producing same
PUBN-DATE: October 21, 1997
INVENTOR-INFORMATION:

NAME	COUNTRY
VOGEL, TIKVA	IL
LEVANON, AVIGDOR	IL
WERBER, MOSHE M	IL
GUY, RACHEL	IL
PANET, AMOS	IL
HARTMAN, JACOB	IL
SHAKED, HADASSA	IL

EUR-CL (EPC): A61K049/00; A61K051/08 ABSTRACT:
CHG DATE=19990617 STATUS=O>This invention provides an imaging agent which comprises a polypeptide labeled with an imageable marker, such polypeptide having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to
fibrin. The invention further provides a method wherein the imaging agent is used for imagifig a fibrin-containing substance, i.e., a thrombus or atherosclerotic plaque. Further provided are plasmids for expression of polypeptides having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin, hosts containing these plasmids, methods of producing the polypeptides, methods of treatment using the polypeptides, and methods of recovering, refolding and reoxidizing the polypeptides. The invention also provides for purified polypeptides substantially free of other substances of human origin which have an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and which are capable of binding to fibrin.
INT-CL_(IPC): A61K051/08
ABSTRACT:
CHG DATE=19990617 STATUS=O>This invention provides an imaging agent which comprises a polypeptide labeled with an imageable marker, such polypeptide having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to
fibrin. The invention further provides a method wherein the imaging agent is used for imagifig a fibrin-containing substance, i.e., a thrombus or atherosclerotic plaque. Further provided are plasmids for expression of polypeptides having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin, hosts containing these plasmids, methods of producing the polypeptides, methods of treatment using the polypeptides, and methods of recovering, refolding and reoxidizing the polypeptides. The invention also provides for purified polypeptides substantially free of other substances of human origin which have an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and which are capable of binding to fibrin.

CLIPPEDIMAGE= US005455158A
PUB-NO: US005455158A
DOCUMENT-IDENTIFIER: US 5455158 A
TITLE: Fibrin binding domain polypeptides and uses and methods of producing same
PUBN-DATE: October 3, 1995
INVENTOR-INFORMATION:

NAME	COUNTRY
VOGEL, TIKVA	IL
LEVANON, AVIGDOR	IL
WERBER, MOSHE M	IL
GUY, RACHEL	IL
PANET, AMOS	IL

EUR-CL (EPC): A61K047/48; A61K049/00, A61K051/08 , A61M025/00 , C07K001/113 , C07K007/06 , C07K014/315 , C07K014/78 , C07K019/00 ABSTRACT:
CHG DATE=19990617 STATUS=O>This invention provides an imaging agent which comprises a polypeptide labeled with an imageable marker, such polypeptide having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to
fibrin. The invention further provides a method wherein the imaging agent is used for imaging a fibrin-containing substance, i.e., a thrombus or atherosclerotic plaque. Further provided are plasmids for expression of polypeptides having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin, hosts containing these plasmids, methods of producing the polypeptides, methods of treatment using the polypeptides, and methods of recovering, refolding and reoxidizing the polypeptides. The invention also provides for purified polypeptides substantially free of other substances of human origin which have an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and which are capable of binding to fibrin.
INT-CL (IPC): G01N033/53; A61K051/08 ; C12Q001/56
ABSTRACT:
CHG DATE=19990617 STATUS=O>This invention provides an imaging agent which comprises a polypeptide labeled with an imageable marker, such polypeptide having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to
fibrin. The invention further provides a method wherein the imaging agent is used for imaging a fibrin-containing substance, i.e., a thrombus or atherosclerotic plaque. Further provided are plasmids for expression of polypeptides having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin, hosts containing these plasmids, methods of producing the polypeptides, methods of treatment using the polypeptides, and methods of recovering, refolding and reoxidizing the polypeptides. The invention also provides for purified polypeptides substantially free of other substances of human origin which have an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and which are capable of binding to fibrin.

CLIPPEDIMAGE= US005270030A
PUB-NO: US005270030A
DOCUMENT-IDENTIFIER: US 5270030 A
TITLE: Fibrin binding domain polypeptide and method of producing
PUBN-DATE: December 14, 1993
INVENTOR-INFORMATION:

NAME	COUNTRY
VOGEL, TIKVA	IL
LEVANON, AVIGDOR	IL
WERBER, MOSHE M	IL
GUY, RACHEL	IL
PANET, AMOS	IL

EUR-CL (EPC): A61K047/48; A61K049/00, A61K051/08 , A61M025/00 , C07K001/113 , C07K007/06 , C07K014/315 , C07K014/78 , C07K019/00 ABSTRACT:
CHG DATE=19990617 STATUS=O>This invention provides an imaging agent which comprises a polypeptide labeled with an imageable marker, such polypeptide having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to
fibrin. The invention further provides a method wherein the imaging agent is used for imaging a fibrin-containing substance, i.e., a thrombus or atherosclerotic plaque. Further provided are plasmids for expression of polypeptides having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin, hosts containing these plasmids, methods of producing the polypeptides, methods of treatment using the polypeptides, and methods of recovering, refolding and reoxidizing the polypeptides. The invention also provides for purified polypeptides substantially free of other substances of human origin which have an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and which are capable of binding to fibrin.
INT-CL (IPC): A61K099/00; C07K013/00 ; C12N015/74 ; C12P021/02
ABSTRACT:
CHG DATE=19990617 STATUS=O>This invention provides an imaging agent which comprises a polypeptide labeled with an imageable marker, such polypeptide having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to
fibrin. The invention further provides a method wherein the imaging agent is used for imaging a fibrin-containing substance, i.e., a thrombus or atherosclerotic plaque. Further provided are plasmids for expression of polypeptides having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin, hosts containing these plasmids, methods of producing the polypeptides, methods of treatment using the polypeptides, and methods of recovering, refolding and reoxidizing the polypeptides. The invention also provides for purified polypeptides substantially free of other substances of human origin which have an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and which are capable of binding to fibrin.

CLIPPEDIMAGE= WO0009117765A1
PUB-NO: WO0009117765A1
DOCUMENT-IDENTIFIER: WO 9117765 A1
TITLE: FIBRIN BINDING DOMAIN POLYPEPTIDES AND USES AND METHODS OF PRODUCING SAME
PUBN-DATE: November 28, 1991
INVENTOR-INFORMATION:

NAME	COUNTRY
VOGEL, TIKVA	IL
LEVANON, AVIGDOR	IL
WERBER, MOSHE	IL
GUY, RACHEL	IL
PANET, AMOS	IL
HARTMAN, JACOB	IL
SHAKED, HADASSA	IL

EUR-CL (EPC): A61K049/00; C07K007/06, A61K047/48 , A61K051/08 , C07K001/113 , C07K014/315 , C07K014/78
US-CL-CURRENT: 435/252

ABSTRACT:
CHG DATE=19990617 STATUS=O>This invention provides an imaging agent which comprises a polypeptide labeled with an imageable marker, such polypeptide having an amino acid sequence substantially present in the fibrin binding domain of naturally-occuring human fibronectin and being capable of binding to fibrin. The invention further provides a method wherein the imaging agent is used for imaging a fibrin-containing substance, i.e., a thrombus or atherosclerotic plaque. Further provided are plasmids for expression of polypeptides having an amino acid sequence substantially present in the fibrin binding domain of naturally-occuring human fibronectin and being capable of binding to fibrin, hosts containing these plasmids, methods of producing the polypeptides, methods of treatment using the polypeptides, and methods of recovering, refolding and reoxidizing the polypeptides. The invention also provides for purified polypeptides substantially free of other substances of human origin which have an amino acid sequence substantially present in the fibrin binding domain of naturally-occuring human fibronectin and which are capable of binding to fibrin.
INT-CL_(IPC): A61K037/10; C07H015/12 ; C12N001/22 ; C12P021/06

US-CL-CURRENT: 435/252

ABSTRACT:
CHG DATE=19990617 STATUS=O>This invention provides an imaging agent which comprises a polypeptide labeled with an imageable marker, such polypeptide having an amino acid sequence substantially present in the fibrin binding domain of naturally-occuring human fibronectin and being capable of binding to fibrin. The invention further provides a method wherein the imaging agent is used for imaging a fibrin-containing substance, i.e., a thrombus or atherosclerotic plaque. Further provided are plasmids for expression of polypeptides having an amino acid sequence substantially present in the fibrin binding domain of naturally-occuring human fibronectin and being capable of binding to fibrin, hosts containing these plasmids, methods of producing the polypeptides, methods of treatment using the polypeptides, and methods of recovering, refolding and reoxidizing the polypeptides. The invention also provides for purified polypeptides substantially free of other substances of human origin which have an amino acid sequence substantially present in the

fibrin binding domain of naturally-occuring human fibronectin and which are capable of binding to fibrin.

DERWENT-ACC-NO: 1991-369004
DERWENT-WEEK: 200146

TITLE: New fibrin binding domain polypeptide(s) - useful in imaging fibrin-contg. substances, to inhibit thrombus formation and treat wounds
INVENTOR-NAME: GUY, R; HARTMAN, J ; LEVANON, A ; PANET, A ; SHAKED, H ; VOGEL, T ; WERBER, M ; WERBER, M M ; VOGEI, T
PRIORITY-DATA: 1990US-0526397 (May 21, 1990) , 1989CA-2006929 (December 29, 1989) , 1993US-0058241 (May 4, 1993) , 1994US-0259569 (June 14, 1994) , 1997US-0826885 (April 8, 1997)
PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
NO 310559 B1	July 23, 2001	N/A	000	C07K 014/745
WO 9117765 A	November 28, 1991	N/A	189	N/A
AU 9180760 A	December 10, 1991	N/A	000	N/A
FI 9205296 A	November 20, 1992	N/A	000	C07K 000/00
NZ 238208 A	January 27, 1993	N/A	000	A61K 049/00
NO 9204405 A	January 13, 1993	N/A	000	C07K 013/00
US 5270030 A	December 14, 1993	N/A	116	A61K 049/00
JP 05508766 W	December 9, 1993	N/A	057	C12P 021/02
HU 66189 T	October 28, 1994	N/A	000	A61K 037/10
EP 651799 A1	May 10, 1995	E	000	C12N 015/12
AU 660618 B	July 6, 1995	N/A	000	C07K 013/00
US 5455158 A	October 3, 1995	N/A	115	G01N 033/53
US 5679320 A	October 21, 1997	N/A	115	A61K 051/08
RU 2109750 C1	April 27, 1998	N/A	000	C07K 014/78
US 5869616 A	February 9, 1999	N/A	000	C07K 014/745
HU 216302 B	June 28, 1999	N/A	000	A61K 038/16
EP 651799 B1	August 18, 1999	E	000	C12N 015/12
DE 69131539 E	September 23, 1999	N/A	000	C12N 015/12
US 5965383 A	October 12, 1999	N/A	000	C12N 015/00
ES 2137928 T3	January 1, 2000	N/A	000	C12N 015/12
US 6121426 A	September 19, 2000	N/A	000	C07K 001/107
KR 219115 B1	September 1, 1999	N/A	000	A61K 051/08

B1

INT-CL (IPC): A61K037/02; A61K037/10 ; A61K038/00 ; A61K038/16 ; A61K038/39 ; A61K047/42 ; A61K049/00 ; A61K049/02 ; A61K051/00 ; A61K051/08 ; C07H015/12 ; C07K000/00 ; C07K001/00 ; C07K001/107 ; C07K003/00 ; C07K003/08 ; C07K003/12 ; C07K013/00 ; C07K014/435 ; C07K014/745 ; C07K014/78 ; C07K015/00 ; C07K015/06 ; C12G000/00 ; C12N001/21 ; C12N001/22 ; C12N005/10 ; C12N009/50 ; C12N009/70 ; C12N009/72 ; C12N015/00 ; C12N015/11 ; C12N015/12 ; C12N015/62 ; C12N015/63 ; C12N015/74 ; C12P021/00 ; C12P021/02 ; C12P021/06 ; C12Q001/56 ; G01N033/53 ; G01N033/68
ABSTRACTED-PUB-NO: EP 651799B
BASIC-ABSTRACT: An imaging agent comprising a polypeptide labelled with a marker is claimed.

The polypeptide has an aminoacid sequence substantially identical to that present in the fibrin-binding domain of naturally-occurring human fibronectin. It binds to fibrin and has a mol. wt. 6-20 kD and the N-terminal sequence

Gln-Ala-Gln-Gln or Met-Gln-Ala-Gln-Gln.

The marker is pref. a radioactive isotope, such as 111In or 99mTc, a paramagnetic ion or an element opaque to x-rays.

Also claimed are a plasmid for expressing the polypeptide, a cell comprising the plasmid, a method of producing the polypeptide and a fusion polypeptide.

USE/ADVANTAGE - The imaging agent is used to image a fibrin-contg. substance such as an atherosclerotic plaque or thrombus. The polypeptide is used to inhibit thrombus formation and to treat wounds e.g. an incision, skin deficit, skin graft, burn, eye wound or a tendon injury.

ABSTRACTED-PUB-NO: US 5270030A
EQUIVALENT-ABSTRACT: An imaging agent comprising a polypeptide labelled with a marker is claimed.

The polypeptide has an aminoacid sequence substantially identical to that present in the fibrin-binding domain of naturally-occurring human fibronectin. It binds to fibrin and has a mol. wt. 6-20 kD and the N-terminal sequence

Gln-Ala-Gln-Gln or Met-Gln-Ala-Gln-Gln.

The marker is pref. a radioactive isotope, such as 111In or 99mTc, a paramagnetic ion or an element opaque to x-rays.

Also claimed are a plasmid for expressing the polypeptide, a cell comprising the plasmid, a method of producing the polypeptide and a fusion polypeptide.

USE/ADVANTAGE - The imaging agent is used to image a fibrin-contg. substance such as an atherosclerotic plaque or thrombus. The polypeptide is used to inhibit thrombus formation and to treat wounds e.g. an incision, skin deficit, skin graft, burn, eye wound or a tendon injury.

Imaging agent comprises a polypeptide (Mr 12,000) having the aminoacid sequence
(units 1-109) of the fibrin-binding domain in naturally occurring human fibronectin, labelled with a radioactive isotope, an element that is opaque to X-rays, or a paramagnetic ion; dispersed with the usual carriers and opt. additives.

USE - The prod. binds to fibrin and facilitates the scanning of fibrinous

zones
(e.g. a thrombus or atherosclerotic plaque) for diagnosis and the monitoring
of
treatment.

A method for imaging a fibrin-containing substance which comprises contacting the fibrin-containing substance to be imaged with an imaging agent which comprises a polypeptide labelled with an imageable marker, such polypeptide having an amino acid sequence substantially identical to a sequence present in the fibrin binding domain of naturally occurring human fibronectin, being capable of binding to fibrin, having a molecular weight above about 6 kD but less than about 20 kD, and having the amino acid sequence gln-ala-gln-gln or met-gln-ala-gln-gln at the N-terminus of the polypeptide under conditions such that the agent binds to the fibrin-containing substance and imaging bound agent
and thereby imaging the fibrin-containing substance.

An imaging agent comprising a polypeptide labelled with a marker is claimed.

The polypeptide has an aminoacid sequence substantially identical to that present in the fibrin-binding domain of naturally-occurring human fibronectin. It binds to fibrin and has a mol. wt. 6-20 kD and the N-terminal sequence

Gln-Ala-Gln-Gln or Met-Gln-Ala-Gln-Gln.

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The marker is pref. a radioactive isotope, such as 111In or 99mTc, a paramagnetic ion or an element opaque to x-rays.

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USE/ADVANTAGE - The imaging agent is used to image a fibrin-contg. substance such as an atherosclerotic plaque or thrombus. The polypeptide is used to inhibit thrombus formation and to treat wounds e.g. an incision, skin deficit, skin graft, burn, eye wound or a tendon injury.

An imaging agent comprising a polypeptide labelled with a marker is claimed.

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Also claimed are a plasmid for expressing the polypeptide, a cell comprising the plasmid, a method of producing the polypeptide and a fusion polypeptide.

USE/ADVANTAGE - The imaging agent is used to image a fibrin-contg. substance such as an atherosclerotic plaque or thrombus. The polypeptide is used to inhibit thrombus formation and to treat wounds e.g. an incision, skin deficit, skin graft, burn, eye wound or a tendon injury.